SUPPLEMENTAL MATERIALS

A short AIP1 isoform localizes to the mitochondria and promotes vascular dysfunction

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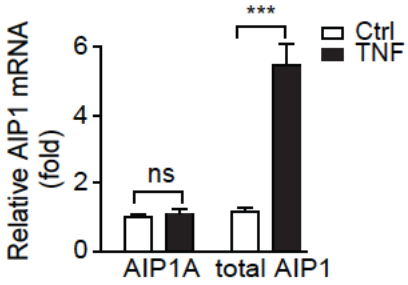
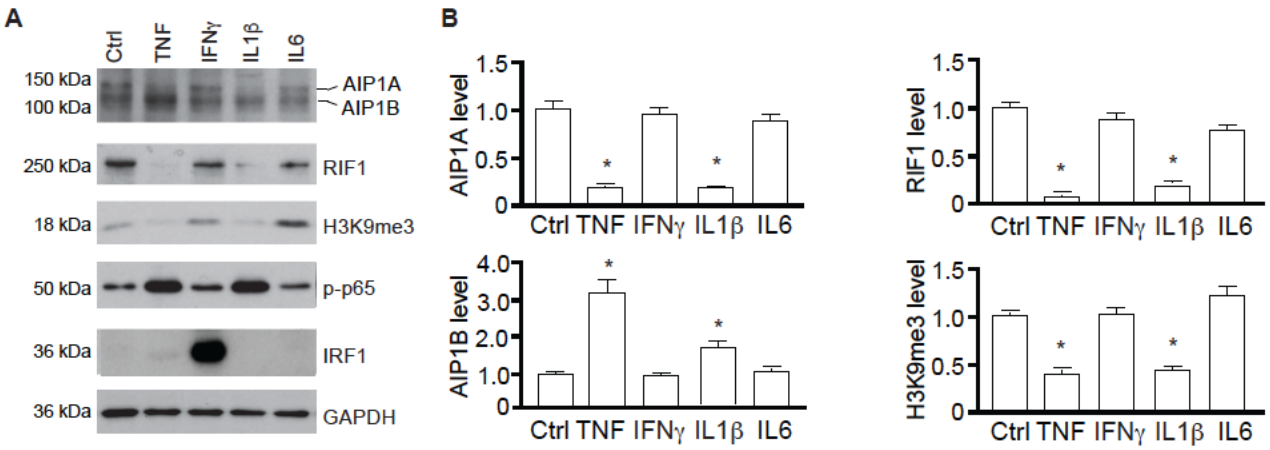
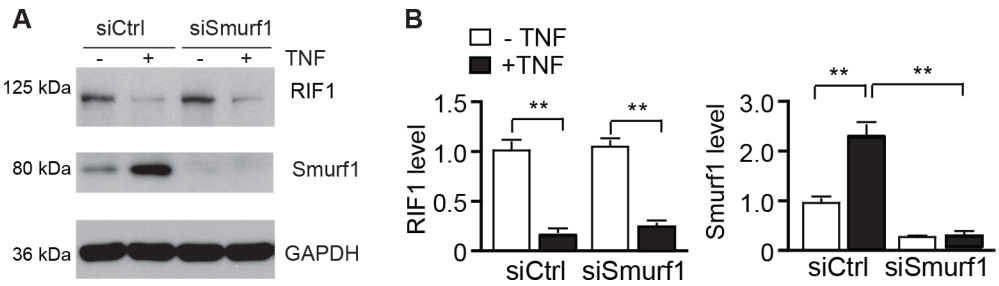
SUPPLEMENTAL FIGURE LEGENDS



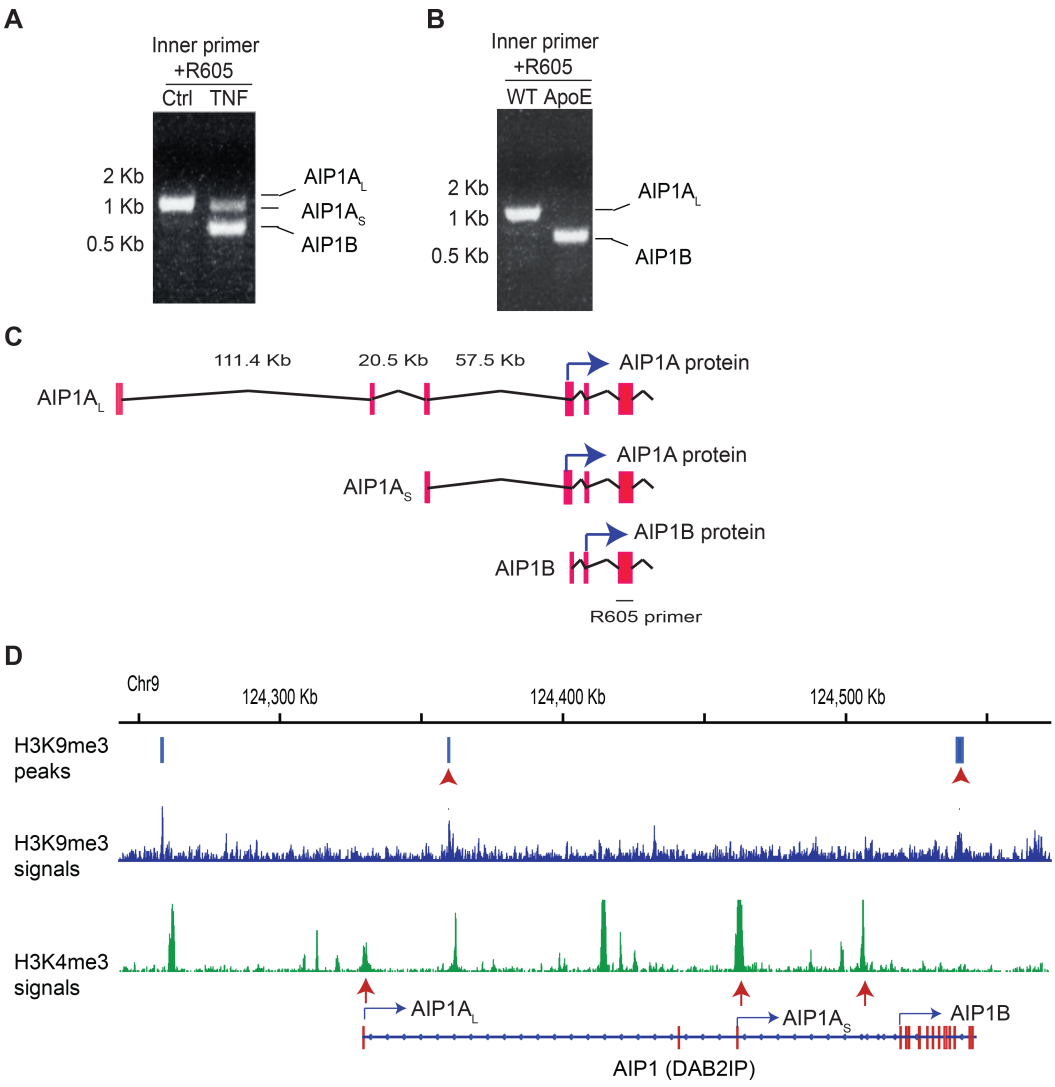
Supplemental Fig.I. Total AIP1 mRNA increased after TNF treatment, but AIP1A mRNA levels were unchanged. HUVECs were treated with control or TNF (10 ng/ml) for 16 h. Then, AIP1A and total AIP1 were measured by RT-PCR with AIP1A 5' UTR-specific and AIP1B (detected both isoforms)-specific primers. Data were normalized to GAPDH expression. The TNF-induced fold changes are presented. n=3. All data are presented as the mean ± standard error of the mean (SEM). ns: non-significant; *** $P<0.001$, unpaired, two tailed *t*-test.



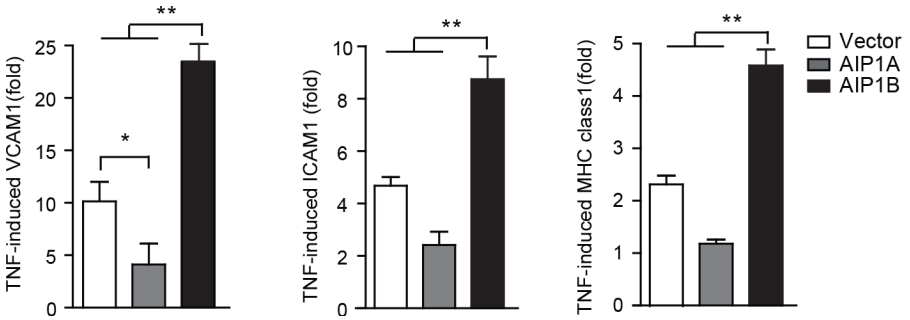
Supplemental Fig.II. Effects of proinflammatory cytokines on AIP1 expression. A. AIP1A, RIF1 and H3K9me3 were downregulated while AIP1B was upregulated in response to TNF. HUVECs were untreated or treated with TNF, IFN-γ, IL-1β or IL-6 (10 ng/ml each) for 24 h. AIP1, RIF1, H3K9me3, TNF-inducible phosphor-NF-κBp65 and IFN-γ-inducible IRF1 were detected by western blotting with respective antibodies. **B.** Protein bands in (A) were quantified by densitometry and fold changes are presented by taking untreated group as 1.0. n=3. All data are presented as the mean ± SEM. * $P<0.05$ comparing cytokine treated group with the control, unpaired, two tailed *t*-test.



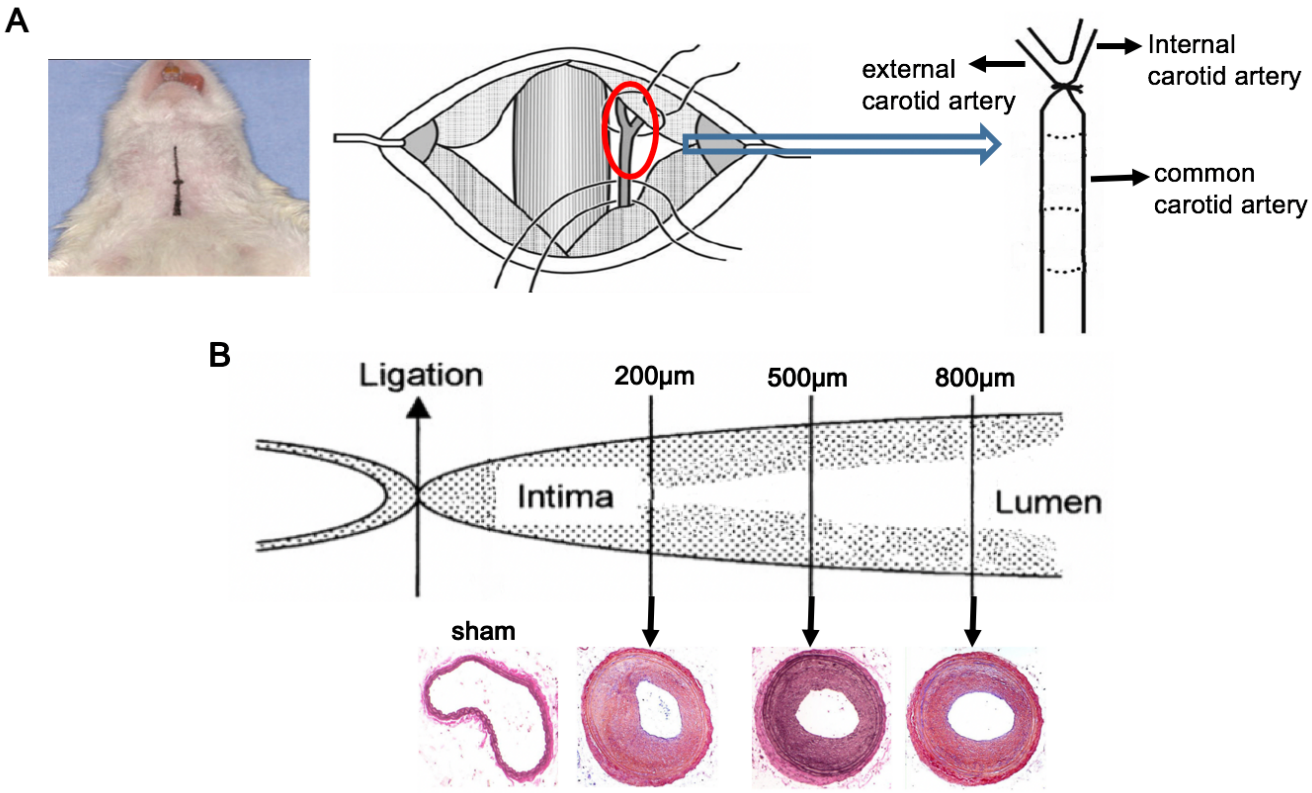
Supplemental Fig.III. TNF-induced RIF1 downregulation is not mediated by Smurf1. **A.** HUVECs were transfected with control or Smurf1 siRNA for 48 h. Cells were then untreated or treated with TNF (10 ng/ml) for 8 h. RIF1, Smurf1, and GAPDH proteins were detected by western blotting. **B.** RIF1 and Smurf1 protein bands in (A) were quantified by densitometry and fold changes are presented by taking untreated group as 1.0. n=3. All data are presented as the mean ± SEM. ** P<0.01, comparing TNF treated group with the control, unpaired, two tailed t-test.



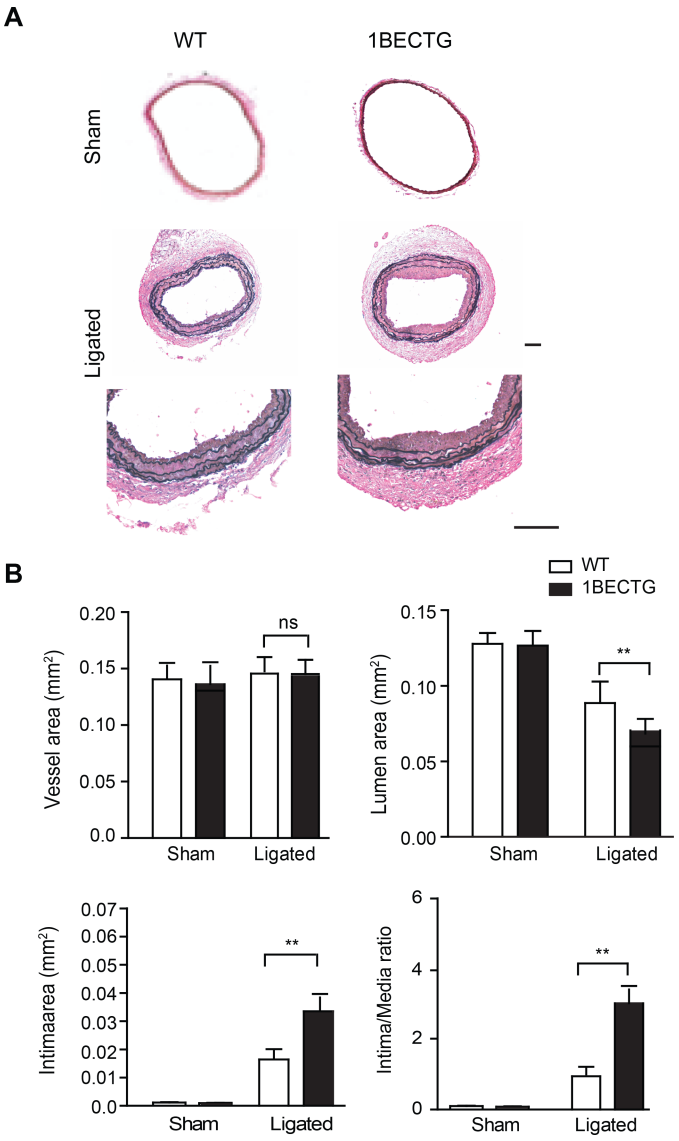
Supplemental Fig.IV. A-B. Expression of the AIP1B transcript was induced in response to TNF in HUVECs and in atherosclerotic aortae. HUVECs were treated with control or TNF (10 ng/ml) for 16 h (A). Aortae from WT and ApoE$^{-/-}$ mice after 20 weeks on a high-fat diet (B). 5' RACE was performed with AIP1-specific nested PCR (human sequence for HUVECs and mouse sequence for mouse aortae) with a set of internal reverse primers (R605). The PCR products were analyzed by agarose gel electrophoresis, followed by extraction for DNA sequencing. n=2. **C**. Diagram of the AIP1 transcripts. Exon sequences from the 5' RACE are depicted in red. Potential translational start sites for the AIP1A and AIP1B proteins are indicated by blue arrows. **D**. Epigenetic regulation of AIP1 in HUVECs. The analyzed H3K9me3 ChIP-seq results in HUVECs were from the ENCODE (Encyclopedia of DNA Elements) and were visualized using the Integrative Genomics Viewer. Two H3K9me3 peaks were identified at the AIP1 locus. One was at the 3' terminus of AIP1, and the other was located around the TSS of AIP1, which is localized at downstream of a H3K4me3 peak (indicated by red arrows) and may act as a potential regulator of the AIP1B promoter. H3K9me3 sites that could suppress AIP1B are indicated by red arrowheads.



Supplemental Fig.V. AIP1A suppressed whereas AIP1B augmented TNF-induced EC activation. HUVECs were transfected with siRNA against the AIP1 3'UTR to knockdown endogenous AIP1 and then infected with lentivirus expressing EGFP (Vector), AIP1A or AIPB. Cells were untreated or treated with TNF (10 ng/ml) for 12 h, and TNF-induced VCAM-1, ICAM-1 and MHC class I expression was measured with RT-PCR that was normalized to GAPDH. TNF-induced fold changes compared to the untreated controls are presented. n=3. All data are presented as the mean ± SEM. ** P<0.01, one-way ANOVA followed by Bonferroni's post-hoc test.



Supplemental Fig.VI. Procedural schematic for the complete ligation of the left common carotid artery (LCA). A. A mouse LCA was completely ligated before the carotid bifurcation of the external, internal carotid, and occipital arteries. The right LCA was un-ligated. **B**. Sections at 200-800 μm from the ligation were examined as illustrated. Examples of sections with vascular remodeling are shown at the bottom.



Supplemental Fig.VII. The AIP1B transgene promoted vascular remodeling in mouse models. Carotid arteries and tissue from WT or AIP1B-ECTG female mice with complete ligation near the carotid bifurcation on the left common carotid artery were harvested at 3 weeks post-ligation. **A**. Histological analysis of artery cross sections with H&E staining. Representative photomicrographs are presented. **B**. Morphometric assessment of the whole vessel (within the EEL), the artery lumen (within the endothelium), the intima (between the endothelium and the IEL), the media (between the IEL and the EEL). The ratio of the intima to the media was calculated from 5 serial cross sections n=6. All data are presented as the mean ± SEM. ns: non-significant; ** $P<0.01$, comparing WT and AIP1BECTG group, unpaired, two tailed *t*-test. Scale bar: 200 μm.

MAJOR RESOURCES TABLES

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
AIP1B-ECTG	Min lab	C57BL/6J	Male /female
ApoE KO	Jax lab 002052	C57BL/6 J	Male /female

Cultured Cells

Name	Vendor or Source	Other Information
HAEC	Lonza	
HUVEC	Yale VBT core	

Antibodies

Target antigen	Vendor/Source	Catalog	Concentration	Lot
AIP1A	Min lab		0.4 μg/ml	
pan-AIP1	Min lab		0.4 μg/ml	
FLAG	Sigma	MAB3118	0.2 μg/ml	
HA	Roche	11 583 816 001	0.2 μg/ml	
GAPDH	Cell signaling technology	2118s	0.5 μg/ml	
RIF1	Abcam	ab13422	0.4 μg/ml	
H3K9me3	Cell signaling technology	13969s	1 μg/ml	
H3K27me3	Cell signaling technology	9733s	1 μg/ml	
H3	Cell signaling technology	4499s	0.2 μg/ml	
Smurf1	Cell signaling technology	2174s	1 μg/ml	
Trx1	Min lab	UR41	0.4 μg/ml	
Trx2	Abcam	ab185544	80 ng/ml	
Tim23	Santa cruz biotechnology	sc-514463	0.4 μg/ml	
p-PERK	Santa cruz biotechnology	sc-32577	0.4 μg/ml	
Golgin-97	Abcam	ab84340	0.4 μg/ml	
β-actin	Sigma	A5441	80 ng/ml	

IκBα	Cell signaling technology	4814s	1 μg/ml	
H3K4me3 (ChIP)	Millipore	07-473	1 μg/ml	
Pol II S5 (ChIP)	Abcam	Ab5131	1 μg/ml	